DEVELOPMENT OF THE EMBRYO SAC AND EMBRYO OF STAPHYLEA TRIFOLIATA.*

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Material for the study of Staphylea trifoliata, L. was collected along the banks of the Olentangy River during several Springs, killed in chrome-acetic acid and imbedded in paraffin. The sections prepared varied in thickness from 8–15 microns. Analin safranin and gentian violet, and iron-alum haematoxylin were used in staining, both giving good results although the latter stain was too dense for pollen grains.

Staphylea trifoliata belongs to the Family Staphyleaceae and to the Order Sapindales and is thus allied to the Hippocastanaceae, Aceraceae, Celastraceae, and Sapindaceae. Scarcely any morphological work seems to have been done on this Order so that very little comparison can be made between Staphylea and nearly related plants. Mottier, Bot. Gaz. **18**:375–377, has reported on the development of the embryo-sac of Acer rubrum and some points of comparison will be noted later. Strasburger also made observations on Staphylea pinnata and Acer in "Zellbildung and Zelltheilung" Jena, 1880; and "Neue Untersuchungen ueber den Befruchtungsvorgang bei den Phanerogamen" Jena 1884.

As a general rule the flowers were quite normal in the number of parts the only variation being four carpels instead of three in the gynoecium. The number of ovules in each carpel may be as high as eight but very rarely more than one matured in each cavity and often only one in the entire capsule. The ovules are anatropous and the best sections were those cut across the ovulary. As soon as the ovules were large enough they were removed from the capsule before killing. The integuments become too woody to make microtome sections long before the embryo is mature. There are two integuments on the ovule but no aril.

The hypodermal archesporial cell (Pl. 19, Fig. 1) appears before there are any traces of integuments. In one case a three celled archesporium (Fig. 2) was found. The single archesporial cell cuts off a primary parietal cell (Fig. 3) which divides to form from three to five tapetal cells (Figs. 4–7) forcing the megasporocyte deep into the tissue of the nucellus. The megasporocyte then divides into four megaspores (Fig. 8) and the lowest becomes functional destroying the others as it enlarges and divides (Figs. 9–10).

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The embryo sac widens slightly as it develops to the eight celled stage (Figs. 11–13) and the nuclei arrange themselves in the normal positions, three at the top becoming the synergidae and egg cell, the three lowest settle into a pocket and gradually disintegrate while the two polar nuclei approach each other and finally come to rest in contact.

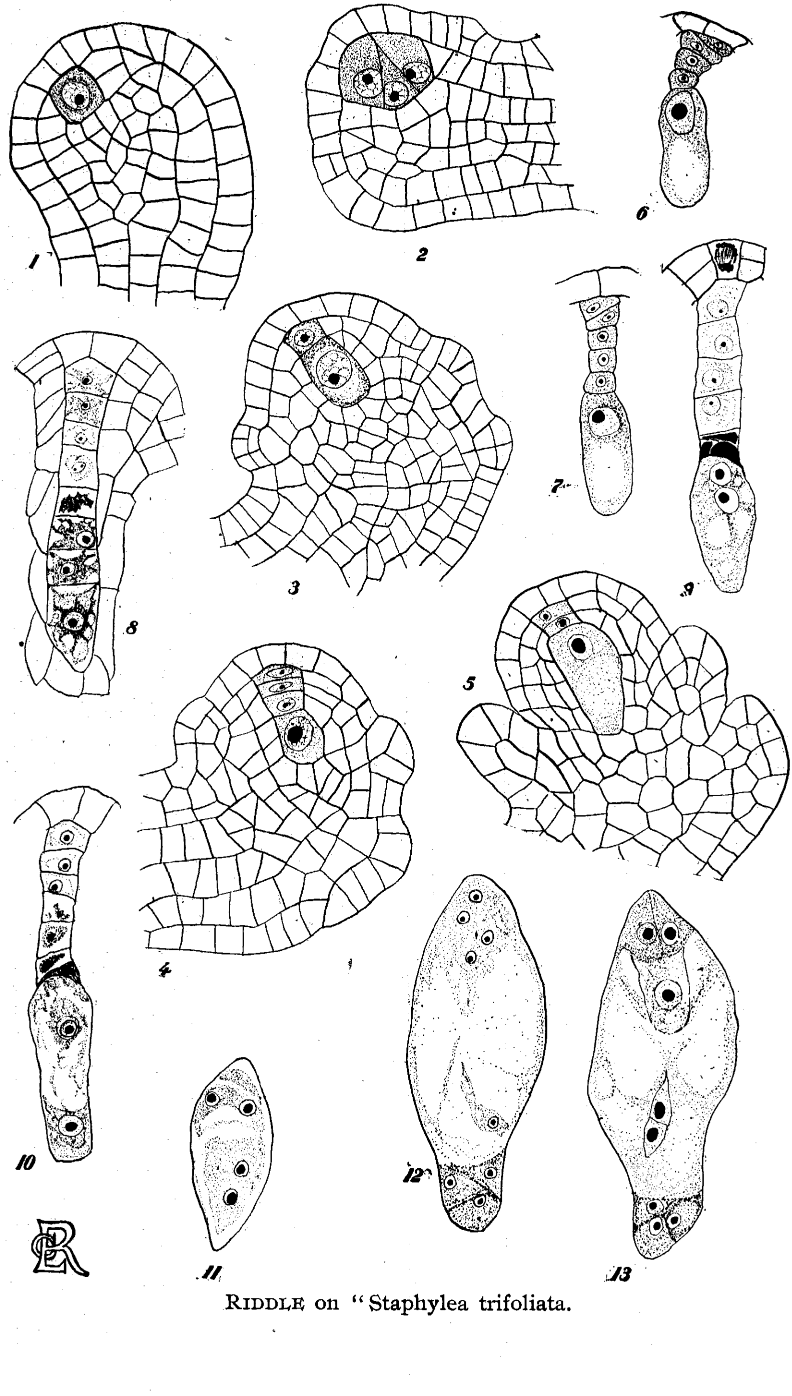
Sections which showed the archesporial cells showed the microspores fully developed and the tapetal layer already breaking down (Fig. 14). Older flowers gave thick-walled pollen grains having two nuclei, the pollen tube nucleus and the generative nucleus. This pollen grain (Fig. 15) resembles those of Acer rubrum (Mottier) and Staphylea pinnata (Strasburger). The latter reports the division of the generative cell into the two sperm cells after the formation of the pollen tube. The generative cell stains quite dark and is apparently enclosed by a wall, making the entire pollen grain very similar to that of the staminate flower of Acer rubrum.

In the formation of the definitive nucleus two polars usually unite (Fig. 13) but in several instances three exactly similar nuclei were found fusing (Fig. 17). In one case, however, there was found what seems to be the union of one of the sperm nuclei with the polars (Fig. 16). This third nucleus is surrounded by a small amount of cytoplasm which stains distinctly darker than that of the polars and the nucleus contains a single small dark nucleolus.

After fertilization the embryo sac enlarges, the formation of endosperm occurs rapidly and the ovule increases greatly in size. The endosperm forms a large loose single layered lining for the entire embryo sac before any division of the one celled embryo occurs. The ovules are about one-fourth the mature size before anything larger than a one-celled embryo is found (Fig. 18). The two-celled (Fig. 19), and four-celled stages (Figs. 20,21 and 25), were found in half grown ovules. Seeds which were full size but still not too hard to section contained embryos still too young to show the development of the cotyledons (Figs. 22–24). Capsules which contained these full sized ovules had attained their normal bladdery inflation. The endosperm was abundant but the nuclei had not begun the formation of walls so that the multinucleate cells which Strasburger finds in Staphylea pinnata were not observed.

The synergidae have usually disappeared or are completely obscured by the abundant endosperm before the one celled embryo divides but in a few cases traces of them were seen with a four-celled embryo (Fig. 20). No traces of the antipodals were evident after division of the endosperm nuclei became rapid.

Division of the suspensor occurs with the formation of the quadrant and seems to retrogress towards the basal cell but the



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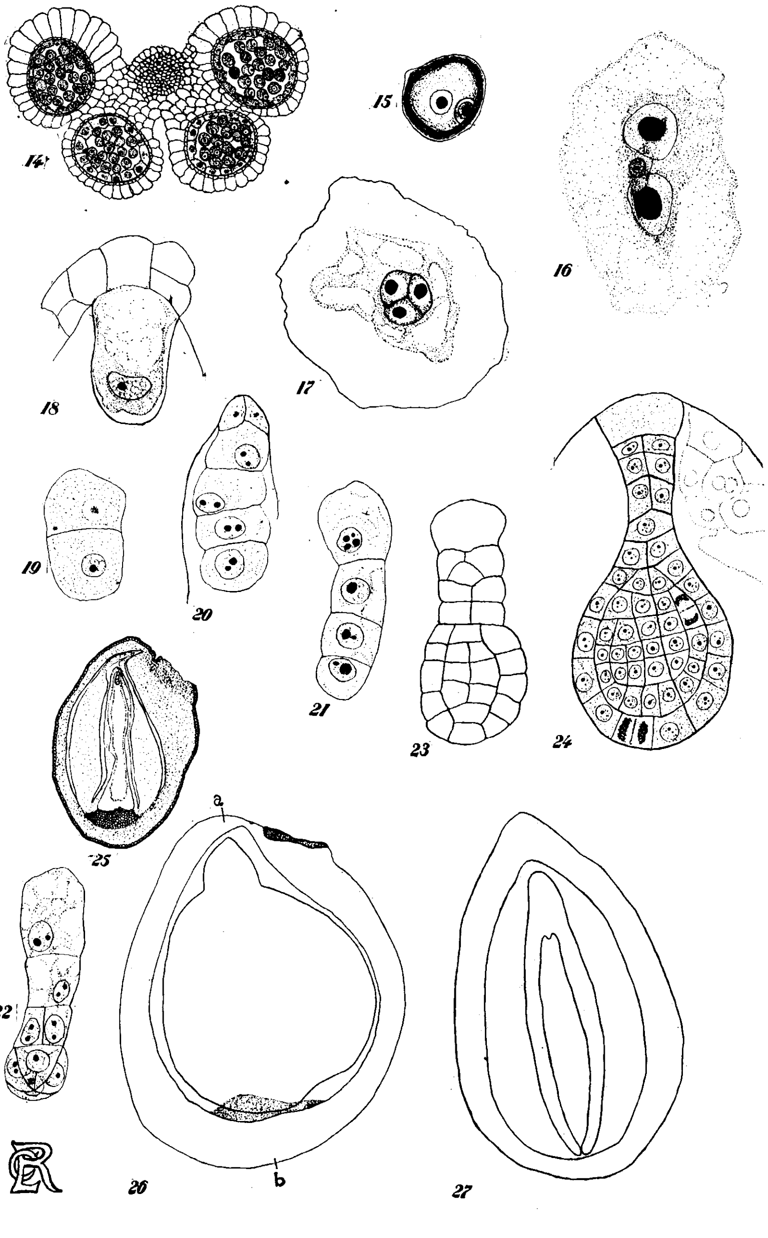

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latter was not seen divided and its nucleus was quite vescicular even in rather young embryos (Figs. 23-24). The abundant endosperm completely surrounds the young embryo which at first develops very slowly. The outer integument becomes very hard, the inner one and the nucellus remaining very spongy and giving way to the growing endosperm. Ovules which contained mature embryos were found in the capsules of the previous year. Hand sections showed a flat straight embryo with two cotyledons (Figs. 26–27). There was no endosperm layer between the cotyledons. The hardening of the outer integument agrees with the observations of Guerin on Staphylea in his study of the seed integuments of some Sapindales. He also notes abundant "albumen" in both Staphylea and Melianthus.

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Explanation of Plates.

For the figures 1–15 a Leitz microscope with No. 6 ocular and No. 7 objective for all but Figure 14 which was drawn with a lower power. A Bausch and Lomb instrument was used for all the other drawings. For Figure 16 the 1-2 ocular and 1-12 oil immersion were used. For Figure 17 the 1-in. ocular and 1-12 objective; for Figures 18–22 the 1-in. ocular and 1-6 objective; for Figures 23–24 a 2-in. ocular and 1-6 objective and for Figures 25–27 the 2-in. ocular and the upper lens of the 2-3 objective.

PLATE XIX.

Fig. 1. Archesporial cell.
Fig. 2. Three celled archesporium.
Fig. 3. Primary parietal cell and megasporocyte. Integuments beginning to develop.
Fig. 4. Three tapetal cells and megasporocyte.
Fig. 5. Two tapetal cells; megasporocyte enlarging.
Figs. 6-7. Four and five tapetal cells.
Fig. 8. Four tapetal cells and four megaspores.

Fig. 9. Four tapetal cells, two celled embryo-sac and disintegrating megaspores.

Fig. 10. Two celled embryo-sac beginning to destroy tapetum.

Fig. 11. Four celled embryo-sac.

Fig. 12. Eight celled embryo-sac showing antipodals already settled in pocket.

Fig. 13. Egg apparatus, conjugating polar nuclei and antipodals.

PLATE XX.

Fig. 14. Stamen showing pollen sacs and pollen grains.

Fig. 15. Older pollen grain with thickened wall.

Fig. 16. Polar nuclei and a sperm nucleus.

Fig. 17. Three nuclei fusing to form definitive nucleus.

Fig. 18. One celled embryo.

Fig. 19. Two celled embryo.

Figs. 20-21. Four celled embryos.

Figs. 22, 23-24. Older embryos.

Fig. 25. Half grown ovule showing four celled embryo, endosperm lining, nucellus and inner integument shrivelling, and outer integument developing hard tissue.

Fig. 26. Flat section of mature seed showing hard integument a-b and outline of embryo imbedded in endosperm.

Fig. 27. Longitudinal section of a mature ovule through a-b showing cotyledons and plumule. Endosperm around but not between cotyledons.